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Effect of Sodium Pentobarbital on Behavioral Thermoregulation in Rats and Mice

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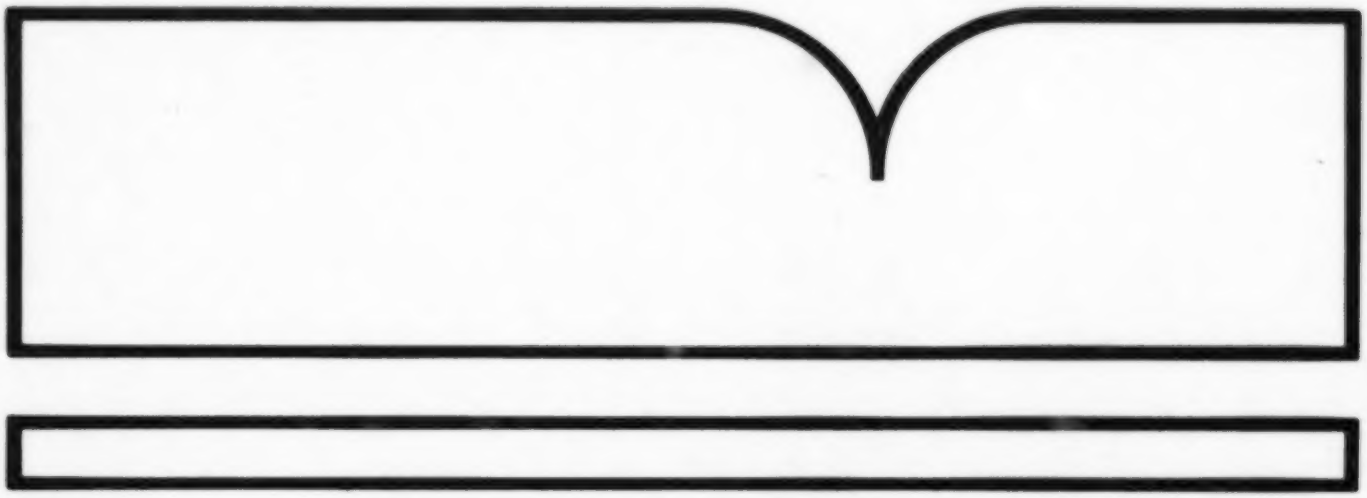

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16. ABSTRACT

This experiment was designed to study the effects of sodium pentobarbital on behavioral thermoregulation in rats and mice. Male Sprague-Dawley rats were given intraperitoneal (i.p.) injections of sodium pentobarbital in doses of 0, 1, 5, 10 or 15 mg/kg and male CBA/J mice were given doses of 0, 5, 10, 15 or 30 mg/kg. The animals were immediately placed in a temperature gradient which allowed them to select their preferred ambient temperature (T_a). The preferred T_a of rats increased following a 10 mg/kg injection of sodium pentobarbital. However, the barbiturate had no effect on the preferred T_a of mice. In another study, male Sprague Dawley rats and CBA/J mice were given i.p. injections of sodium pentobarbital in doses of 0, 5, 10 and 15 mg/kg and then placed into a temperature-controlled environmental chamber set at 30° C for mice and 25 °C for rats (i.e., their preferred T_a when dosed with sodium pentobarbital). Colonic temperatures were taken one hour after injection. Sodium pentobarbital induced dose dependent hypothermia in rats at 25 °C and hyperthermia in mice at 30 °C. These data suggest a peripheral action of sodium pentobarbital in rats which results in hypothermia and an appropriate compensatory behavioral selection of a warmer T_a.

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BRIEF COMMUNICATION

Effect of Sodium Pentobarbital on Behavioral Thermoregulation in Rats and Mice[1]

KIMI S. STREK,[2] MERRITT D. LONG AND CHRISTOPHER J. GORDON[3]

Experimental Biology Division, Health Effects Research Laboratory
U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

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STREK, K. S., M. D. LONG AND C. J. GORDON. *Effect of sodium pentobarbital on behavioral thermoregulation in rats and mice*. PHARMACOL BIOCHEM BEHAV 24(4) 1147–1150, 1986.—In this study on behavioral thermoregulation, male Sprague-Dawley rats were given intraperitoneal (IP) injections of sodium pentobarbital in doses of 0, 1, 5, 10 or 15 mg/kg and male CBA/J mice were given doses of 0, 5, 10, 15 or 30 mg/kg. The animals were immediately placed in a temperature gradient which allowed them to select their preferred ambient temperature (T_a). The preferred T_a of rats increased following an injection of 10 mg/kg sodium pentobarbital, whereas, the barbiturate had no effect on the preferred T_a of mice. In another study, male rats and mice were given sodium pentobarbital in doses of 0, 5, 10 and 15 mg/kg and then placed into a temperature-controlled environmental chamber set at 30°C for mice and 25°C for rats (i.e., their approximate preferred T_a when dosed with sodium pentobarbital). Colonic temperatures were taken one hour after injection. Sodium pentobarbital induced dose dependent hypothermia in rats at 25°C and hyperthermia in mice at 30°C. These data suggest a direct or indirect block of heat gain/conserving effectors in rats treated with sodium pentobarbital which results in hypothermia and an appropriate compensatory selection of a warmer T_a.

Behavioral thermoregulation Sodium pentobarbital Rats Mice Temperature gradient

SODIUM pentobarbital has been reported to affect various components of the thermoregulatory system such as metabolic rate and vasodilatory control [8, 9, 10]. However, there is little information on the effects of subanesthetic doses of sodium pentobarbital on behavioral temperature regulation. Weiss and Laties [13] found that injections of sodium pentobarbital in rats affected operant behavioral thermoregulation by promoting a depression in heat reinforcement rate when rats were allowed to control a heat lamp. Generally, barbiturates reversibly depress activity of excitable tissues [6]. However, it is not clear whether sodium pentobarbital-induced thermoregulatory effects in various species involve a direct effect on the central nervous system (e.g., affect set point) or whether the effects are attributable to the depression of the thermoregulatory effectors. Intravenous injection of sodium pentobarbital suppresses the firing rate of thermal sensitive neurons in the brainstem [11]. This suggests the possibility of a direct central effect of the barbiturate on the control of behavioral and autonomic thermoregulatory effectors.

There are two general ways in which body temperature changes could occur due to the effects of a drug such as sodium pentobarbital [1, 2, 12]; (1) the drug displaces the set-point by directly affecting activity in the central nervous system or by altering afferent inputs, or (2) it directly affects the activity of the thermoregulatory effectors. A generally accepted method of determining whether a drug is causing a set-point displacement or acting on effector mechanisms is by assessing its effects on behavioral thermoregulation [12]. If the agent is causing a set-point displacement, the animal should compensate behaviorally to try to defend that new set-point level, (e.g., a drug resulting in hypothermia would lead to the animal's preference for a lower ambient temperature). Conversely, if the drug acts on the effector mechanisms without altering the set-point, the animal would behaviorally try to counter the change, (e.g., a hypothermic

[1]This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names of commercial products does not constitute endorsement or recommendation for use.

[2]Formerly K. S. Fehlner.

[3]Requests for reprints should be addressed to C. J. Gordon.

drug would lead to an increase in preferred T_a). A temperature gradient provides an effective measure of behavioral temperature regulation by allowing the animal to select its own preferred T_a. This study was designed to assess the effects of sodium pentobarbital on behavioral thermoregulation of both rats and mice.

METHOD

Thirty-seven male rats of the Sprague-Dawley strain with a mean body weight of 383.3±55.9 (S.D.) and fifty-eight male mice of the CBA/J strain with mean body weight of 27.9±1.9 g (S.D.) were used in these studies. The animals were housed in temperature-controlled rooms (22±1.0°C) and kept under standard lighting conditions (12:12 L:D) with a relative humidity of 50%. Food (Purina rat or mouse chow) and water were provided ad lib.

Drug Preparation

Solutions of sodium pentobarbital were made from commercially-prepared 50 mg/ml aliquots (Nembutal, Abbott Laboratories, Chicago, IL) by dilution with 0.9% saline into volumes of 0.1 ml/100 g for injection in rats and 0.5 ml/100 g for injection in mice. Five dose levels of 1.0, 5.0, 10.0, 15.0 and 30.0 mg/kg, and a saline control, were prepared in autoclaved serum vials. All solutions were filtered through a 0.2 μ filter (Gelman, product No. 4192) prior to use to remove extraneous pyrogens.

Apparatus

Preferred ambient temperature (T_a) of unrestrained mice was measured using an automated temperature gradient system previously described [3]. Briefly, the system consisted of an aluminum tube positioned between a warm (43°C) and cool (14°C) water bath. Dry air was blown into the system at a flow rate of ~1.0 l/min.

A similarly-designed but larger temperature gradient system was used to measure preferred T_a of the rats. This system was made from 1.27 cm thick aluminum and had interior dimensions of 231.4 (length) × 12.7 (width) × 11.4 (height) cm. The ends of the gradient were maintained at 47° and 4°C, which resulted in floor temperatures ranging from 11.7°C to 43°C. Floor temperature was found to be better correlated with position in the gradient. A 12-mm slot down the length of the gradient permitted exchange of air in the gradient. Both the rat and the mouse systems utilized a phototransistor-based detection system to continuously record position of the animals [3].

Protocol

Preferred temperature. Naive rats were injected intraperitoneally (IP) with 1.0, 5.0, 10.0 or 15.0 mg/kg sodium pentobarbital or saline vehicle and were placed in the gradient for 2 hours while preferred T_a was continuously monitored. Mice were given an IP injection of sodium pentobarbital at doses of 5.0, 10.0 or 15.0 mg/kg or saline vehicle and were likewise placed into the temperature gradient for two hr while position was continuously recorded. At the end of the 2 hr period, the animals were removed and the gradients were cleaned with soapy water, rinsed with water and swabbed with alcohol to eliminate any olfactory cues which might alter behavioral responses in subsequent experiments. Each animal was used only once.

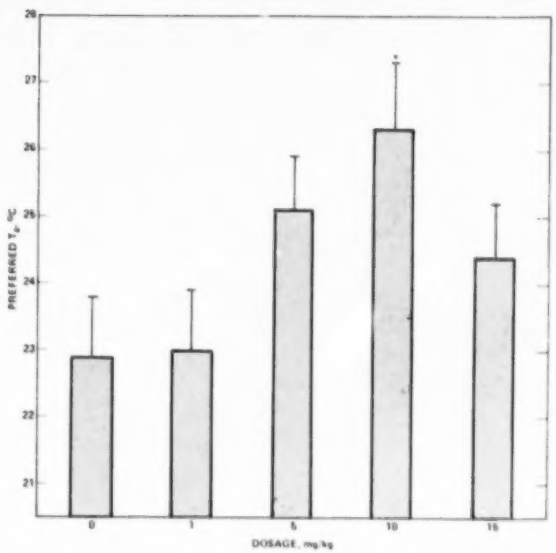

FIG. 1. Mean preferred ambient temperature (T_a), averaged over a 2-hr period, ±S.E.M. of rats (n=5/dose) injected IP with sodium pentobarbitol. An asterisk (*) indicates a significant difference in preferred T_a from 0 mg/kg at $p<0.05$.

Colonic temperature measurements. In another experiment, rats and mice were given 0, 5.0, 10.0, or 15.0 mg/kg of sodium pentobarbital and placed into temperature-controlled environmental chambers for one hour. The rats were tested at a T_a of 25°C whereas the mice were tested at 30°C. These T_as were selected following analysis of the behavioral data and approximated the preferred T_a of each species following pentobarbital injection (see below). After one hour, colonic temperatures were taken by inserting thermistor probes 6.4 and 2.4 cm past the anal sphincter of the rats and mice, respectively. Temperature measurements were rapidly obtained in order to reduce any artifactual rise in colonic temperature due to manipulation of the animals and possible stress. It should be noted that the environmental chambers are immersed in a temperature-controlled water bath. In this system of temperature control, floor temperature is nearly equal to air temperature which facilitates comparison of these data to that collected from the temperature gradient.

Statistics

Preferred temperature measurements were analyzed by determining the mean preferred T_a at twenty minute intervals and then compiling the resulting means into a single value. Student's *t*-test was used to determine whether there were any significant effects of sodium pentobarbital on preferred T_a of each species. Linear regression analysis was used to assess any changes in colonic temperature as a function of dose of pentobarbital.

RESULTS

Rats treated with saline selected a T_a of 22.9°C when averaged over a two hour period (Fig. 1). Sodium pentobar-

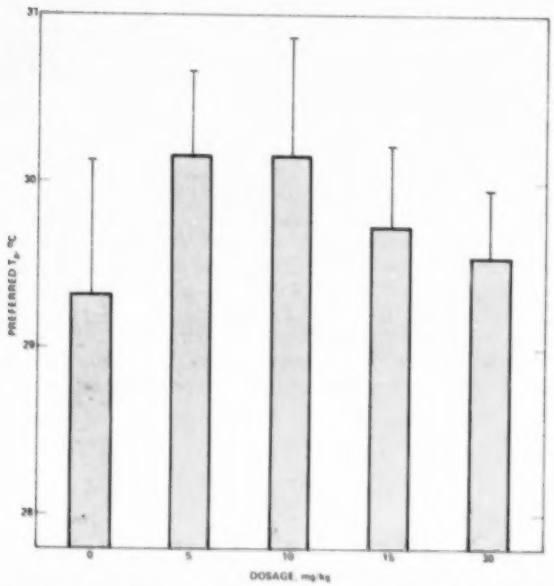

FIG. 2. Mean preferred T_a, averaged over a 2-hr period, (±S.E.M.) of mice (n=7/dose) injected IP with sodium pentobarbital. An additional test with 30 mg/kg was run for 1.5 hr (n=14).

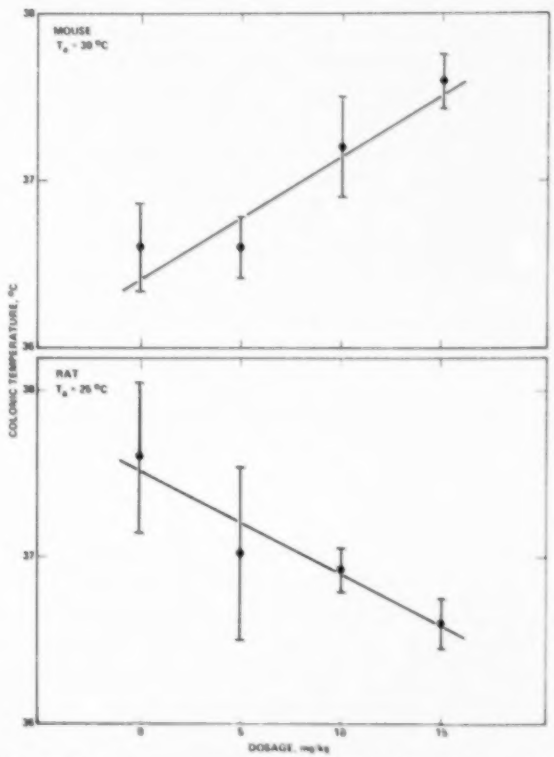

FIG. 3. Colonic temperature of mice at 30°C (n=16) and rats at 25°C (n=12) 1 hr after injection of sodium pentobarbital. Vertical bars represent ±S.E.M. Linear regression analysis yielded: y = 0.0725x + 36.413 and r=0.69 for mice; y = −0.062x + 37.507 and r=0.56 for rats. Probability that slopes are not equal to zero is <0.05 for both mice and rats.

bital at a dose of 10 mg/kg induced a slight but statistically significant ($p<0.05$) increase in preferred T_a. Doses of 5.0 and 15.0 mg/kg sodium pentobarbital did not significantly elevate preferred T_a.

Mice treated with saline selected a T_a of 29.3°C when averaged over a two-hour period (Fig. 2). No significant change in preferred T_a was detected at sodium pentobarbital doses of 5.0 to 15.0 mg/kg. In a followup study, preferred T_a was measured for 90 min in CBA/J mice injected with 30 mg/kg sodium pentobarbital. Even at this high dose there was no change in behavioral thermal preference (Fig. 2).

Colonic temperature following sodium pentobarbital treatment at each species' preferred T_a (i.e., rat ~25°C, mouse ~30°C) was quite variable. In rats there was a significant decrease ($p<0.05$) in colonic temperature with increasing dose of sodium pentobarbital (Fig. 3). Conversely, in mice there was a significant increase ($p<0.05$) in colonic temperature with increasing dose. For example, at a dose of 15 mg/kg, colonic temperature of rats decreased by 1.0°C whereas, for mice, colonic temperature increased by 1.0°C (Fig. 3).

DISCUSSION

The effects of sodium pentobarbital on behavioral thermoregulation are subtle and species dependent. For example, in rats, there was a significant increase in preferred T_a from 23 to 26°C at a dose of 10 but not 15 mg/kg. Based on the colonic temperature experiments it would appear that these animals were slightly hypothermic when treated with the aforementioned doses of sodium pentobarbital (Fig. 3). On the other hand, mice exhibited no change in their preferred T_a of approximately 30°C at doses as high as 30 mg/kg in spite of the fact that, at this T_a, these animals were slightly hyperthermic following sodium pentobarbital injection (e.g., Fig. 3). It is not clear why a 15 mg/kg dose in the rat had no significant effect on preferred T_a. At this dose rats did display a reduction in movement in the gradient. It is possible that this high dose resulted in an anesthetic action which impaired behavioral temperature selection.

In a study by Lin [8], IP injections of sodium pentobarbital were found to lower colonic temperature in rats at a T_a of 8° and 22°C, as we observed in the present study at 25°C. Sodium pentobarbital-induced hypothermia in rats results from a decreased metabolic rate and peripheral vasodilation [8]. Furthermore, the effect of sodium pentobarbital on thermoregulation may be attributable to the activation of serotonin release in the CNS [8]. The behavioral data from the present study demonstrated selection of a warmer T_a following pentobarbital injection in the rat and suggests an action of sodium pentobarbital on the effector mechanisms (e.g., a forced hypothermia, see introduction). That is, control of behavioral thermoregulation was unaffected and the sodium pentobarbital-treated rats attempted to behaviorally compensate for the hypothermia. Results from operant behavioral assessments after IP sodium pentobarbital injection in rats [13] showed no change in behavioral reinforcement for heat at 5 and 10 mg/kg but a depression for heat reinforcement at 20 mg/kg and severe hypothermia. Similarly, in

our study on rats, a high 15 mg/kg dose of sodium pentobarbital failed to elicit a significant increase in preferred T_a in spite of the large decrease in colonic temperature.

Humphreys *et al.* [7] studied the effects of sodium pentobarbital microinjected into specific brain sites of the rat. Their results were opposite to that of our study. For example, microinjection of sodium pentobarbital into the POAH (preoptic area/anterior hypothalamus) resulted in significant hyperthermia at a T_a of 23°C but only slight hyperthermia at 10 and 34°C. Operant assessments of behavioral thermoregulation utilizing a system in which the rats pressed a lever to shut off a 250 W lamp showed a decrease in the amount of time spent pressing the lever to escape heat.

The behavioral and colonic temperature response of rats and mice to sodium pentobarbital differed considerably. The mice became hyperthermic with essentially no change in preferred T_a while rats became hypothermic at a slightly elevated preferred T_a following sodium pentobarbital injection. Although it is possible that mice and rats have different autonomic and behavioral mechanisms in response to sodium pentobarbital, the fact that mice preferred a much warmer T_a (30°C) than that of the rats (23°C) may explain the apparent different behavioral responses of the two species to sodium pentobarbital.

In a related series of studies, our laboratory has recently reported that mice injected IP with sublethal doses of toxic chemicals such as sulfolane [5] and triethyltin [4] undergo a reduction in preferred T_a along with severe hypothermia. It was thought that this toxic effect may be due to an anesthetic type action of the chemical agent on the central nervous system. However, the data from the present study which showed no effect of sodium pentobarbital on the temperature preference of mice suggests that the mechanism of action of chemical toxicants on temperature regulation in mice may be completely different from that of sodium pentobarbital.

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